Band 5: A Human Testis Specific Protein

Cross Reference to Relation Applications

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/527,875 filed on December 8, 2003.

Statement Regarding Federally Sponsored Research or Development

This invention was made with United States Government support under Grant Nos. HD 38082, and U54 29099, awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

Background

Lipid raft domains are regions of plasma membranes that have distinct lipid content and are enriched in cholesterol and sphingolipids. The unique content of these domains is believed to recruit specific proteins to the plasma membrane and these domains are implicated in signal transduction. If the protein caveolin is present then the membrane domain is defined as a caveolae. Caveolins are cholesterol binding proteins that can potentially regulate a variety of signal transduction pathways (Smart et al., (1999) Mol. Cell. Biol. 19, 7289-7304; Kurzchalia & Parton, (1999) Curr. Opin. Cell. Biol. 11, 424-431). Additional uncharacterized proteins are believed to be associated with the lipid raft domains, and since the raft domain dissociates with capacitation these proteins may play key roles in the capacitation process.

After ejaculation, sperm are able to move actively but lack fertilizing competence. They acquire the ability to fertilize in the female genital tract in a time-dependent process called capacitation. Capacitation has been demonstrated to be accompanied by phosphorylation of several proteins on both serine/threonine and tyrosine residues, and that protein tyrosine phosphorylation is regulated downstream by a cAMP/PKA pathway that involves the crosstalk between these two signaling pathways. With the exception of PKA, the other kinase(s) involved in the regulation of capacitation are still unknown.

Raft fractions can be isolated with reproducibility from mouse caudal sperm. The purified proteins present fractions of the isolated lipid raft domains isolated from noncapacitated sperm differ from those isolated from capacitated sperm.

There is a long felt need in the art for better methods to identify testis and sperm specific proteins for diagnostic and therapeutic purposes. There is also a need in the art for methods of contraception based on targeting testis and sperm specific proteins. The present invention satisfies these needs.

Summary of Various Embodiments of the Invention

The present invention is directed to mammalian Band 5 genes and proteins, homologs, fragments, derivatives and fragments, thereof, isolated nucleic acids encoding the respective proteins, and antibodies against those proteins. The present invention is also directed to identifying and using agents which regulate Band 5 gene and protein expression and function.

In accordance with one embodiment of the present invention a sperm raft domain associated protein is isolated and characterized. More particularly, the present invention is directed to polypeptides comprising the amino acid sequences disclosed in Figure 6 (SEQ ID NOs:7 and 8) and to homologs, fragments, derivatives and modifications thereof. Antagonists of Band 5 activity are anticipated to have utility as contraceptive agents and thus one aspect of the present invention is directed to a method of screening for regulators of Band 5 gene and protein expression and activity. In accordance with one embodiment, the human and mouse Band 5 genes and proteins serve as targets for the development of novel drugs, including the identification of novel contraceptive agents. In one aspect, the agent which regulates Band 5 is useful as a contraceptive. In one embodiment, the invention provides a method to inhibit Band 5 gene and protein expression and activity. The present invention also encompasses antibodies specific for Band 5 and the use of such antibodies as therapeutic and diagnostic tools. The invention also encompasses the use of antisense oligonucleotides as therapeutic and contraceptive agents.

Brief Description of the Drawings

The foregoing summary, as well as the following detailed description of preferred embodiments of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiment which are presently preferred. It should be understood, however, that the invention is not limited to the

precise arrangements and instrumentalities shown. In the drawings:

Figure 1 represents a schematic diagram of the preparation of protein fractions as described herein.

Figure 2 represents an electrophoretic analysis of RAFT isolation fractions from noncapacitated and capacitated mouse sperm.

Figure 3 depicts the results the initial mass spec identification of Band 5 proteins and sequences used in the identification procedure.

Figure 4 represents a bioinformatics summary of protein and nucleotide Blasts for human and mouse Band 5 proteins.

Figure 5 represents a schematic protein model based on bioinformatic information.

Figure 6 represents a pair wise alignment of mouse (SEQ ID NO:7) and human (SEQ ID NO:8) protein amino acid sequences.

Figure 7, comprising Figures 7A and 7B, represents PCR analyses of mouse testis cDNA. Figure 7A is a graphic representation of the threshold cycle according to the PCR cycle where product fluorescence crosses the threshold. Figure 7B is a graphic representation of a melt cure indicating the temperature at which the product melts.

Figure 8 is a graphic representation of real-time PCR for a mouse multiple tissue cDNA panel screen. The ordinate represents PCR Base Line Subtracted RFU and the abscissa represents threshold cycle.

Figure 9, comprising panels 9A and 9B, represents an electrophoretic analysis and comparison of anti-peptide antibody.

Figure 10 represents an electrophoretic analysis of tyrosine phosphorylation of sperm proteins as markers of capacitation.

Detailed Description of Embodiments

Definitions

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element. "Plurality" means at least two.

As used herein, the term "antibody" refers to a polyclonal or monoclonal antibody or a binding fragment thereof such as Fab, F(ab')2 and Fv fragments. By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

"Antisense" refers particularly to the nucleic acid sequence of the non-coding strand of a double stranded DNA molecule encoding a protein, or to a sequence which is substantially homologous to the non-coding strand. As defined herein, an antisense sequence is complementary to the sequence of a double stranded DNA molecule encoding a protein. It is not necessary that the antisense sequence be complementary solely to the coding portion of the coding strand of the DNA molecule. The antisense sequence may be complementary to regulatory sequences specified on the coding strand of a DNA molecule encoding a protein, which regulatory sequences control expression of the coding sequences.

As used herein, the term "antisense oligonucleotide" means a nucleic acid polymer, at least a portion of which is complementary to a nucleic acid which is present in a normal cell or in an affected cell. The antisense oligonucleotides of the invention preferably comprise between about fourteen and about fifty nucleotides. More preferably, the antisense oligonucleotides comprise between about twelve and about thirty nucleotides. Most preferably, the antisense oligonucleotides comprise between about sixteen and about twenty-one nucleotides. The antisense oligonucleotides of the invention include, but are not limited to, phosphorothioate oligonucleotides and other modifications of oligonucleotides. Methods for synthesizing oligonucleotides, phosphorothioate oligonucleotides, and otherwise modified oligonucleotides are well known in the art (U.S. Patent No: 5,034,506; Nielsen et al., 1991, Science 254: 1497).

As used herein, the term "biologically active fragments" or "bioactive fragment" of a tssk polypeptide encompasses natural or synthetic portions of the full-length protein that are capable of specific binding to their natural ligand.

"Complementary" refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 50%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (e.g., A:T and G:C nucleotide pairs). It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is antiparallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is antiparallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. Preferably, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and preferably at least about 75%, at least about 90%, or at least about 95% of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. More preferably, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

A "control" cell is a cell having the same cell type as a test cell. The control cell may, for example, be examined at precisely or nearly the same time the test cell is examined. The control cell may also, for example, be examined at a time distant from the time at which the test cell is examined, and the results of the examination of the control cell may be recorded so that the recorded results may be compared with results obtained by examination of a test cell.

As used herein, the term "conservative amino acid substitution" is defined herein as an amino acid exchange within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:

Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides:
Asp, Asn, Glu, Gln;
III. Polar, positively charged residues:
His, Arg, Lys;
IV. Large, aliphatic, nonpolar residues:
Met Leu, Ile, Val, Cys
V. Large, aromatic residues:
Phe, Tyr, Trp

A "first nucleic acid region" and a "second nucleic acid region" are "arranged in an antiparallel fashion" if, when the first region is fixed in space and extends in a direction from its 5'-end to its 3'-end, at least a portion of the second region lies parallel to the first strand and extends in the same direction from its 3'-end to its 5'-end.

"Homologous" as used herein, refers to the subunit sequence similarity between two polymeric molecules, e.g., between two nucleic acid molecules, e.g., two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the positions, e.g., 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences 3'ATTGCC5' and 3'TATGGC share 50% homology.

As used herein, "homology" is used synonymously with "identity."

As used herein "an inhibitor of Band 5 activity" or "Band 5 inhibitor" is intended to include any compound, composition or environmental factor that interacts with the Band 5 protein and decreases capacitation-associated tyrosine phosphorylation of sperm proteins, or interacts with Band 5 protein and decreases contraception. An inhibitor of Band 5 also includes inhibitors which inhibit expression of Band 5 nucleic acids and proteins.

As used herein, "Band 5 protein" refers to Band 5 proteins described herein, as well as homologs, derivatives, and fragments thereof.

An "isolated nucleic acid" refers to a nucleic acid segment or fragment which has been separated from sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, e.g., the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to nucleic acids which have been substantially purified from other components which naturally accompany the nucleic acid, e.g., RNA or DNA or proteins, which naturally accompany it in the cell. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., as a cDNA or a genomic or cDNA fragment produced by PCR or restriction enzyme digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

A "ligand" is a compound that specifically binds to a target receptor or molecule.

A "receptor" is a compound that specifically binds to a ligand.

A ligand or a receptor (e.g., an antibody) "specifically binds to" or "is specifically immunoreactive with" a compound when the ligand or receptor functions in a binding reaction which is determinative of the presence of the compound in a sample of heterogeneous compounds. Thus, under designated assay (e.g., immunoassay) conditions, the ligand or receptor binds preferentially to a particular compound and does not bind in a significant amount to other compounds present in the sample. For example, a polynucleotide specifically binds under hybridization conditions to a compound polynucleotide comprising a complementary sequence; an antibody specifically binds under immunoassay conditions to an antigen bearing an epitope against which the antibody was raised. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically

The term "non-native promoter" as used herein refers to any promoter that has been operably linked to a coding sequence wherein the coding sequence and the

promoter are not naturally associated (i.e. a recombinant promoter/coding sequence construct).

As used herein, "nucleic acid," "DNA," and similar terms also include nucleic acid analogs, i.e. analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention.

By "nucleic acid" is also meant any nucleic acid, whether composed of deoxyribonucleosides or ribonucleosides, and whether composed of phosphodiester linkages or modified linkages such as phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphoramidate, bridged phosphoramidate, bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages. The term nucleic acid also specifically includes nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil).

"Operably linked" refers to a juxtaposition wherein the components are configured so as to perform their usual function. Thus, control sequences or promoters operably linked to a coding sequence are capable of effecting the expression of the coding sequence.

A "pathoindicative" cell is a cell which, when present in a tissue, is an indication that the animal in which the tissue is located (or from which the tissue was obtained) is afflicted with a disease or disorder.

The term "peptide" encompasses a sequence of 3 or more amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. Peptide mimetics include peptides having one or more of the following modifications:

1. peptides wherein one or more of the peptidyl --C(O)NR-- linkages (bonds) have been replaced by a non-peptidyl linkage such as a --CH_2-carbamate linkage

(--$CH_2OC(O)NR$--), a phosphonate linkage, a -CH_2-sulfonamide (-CH_2--$S(O)_2$ NR--) linkage, a urea (--NHC(O)NH--) linkage, a --CH_2 -secondary amine linkage, or with an alkylated peptidyl linkage (--C(O)NR--) wherein R is C_1-C_4 alkyl;

2. peptides wherein the N-terminus is derivatized to a --NRR_1 group, to a -- NRC(O)R group, to a --NRC(O)OR group, to a --$NRS(O)_2R$ group, to a --NHC(O)NHR group where R and R_1 are hydrogen or C_1-C_4 alkyl with the proviso that R and R_1 are not both hydrogen;

3. peptides wherein the C terminus is derivatized to --$C(O)R_2$ where R_2 is selected from the group consisting of C_1-C_4 alkoxy, and --NR_3R_4 where R_3 and R_4 are independently selected from the group consisting of hydrogen and C_1-C_4 alkyl.

Naturally occurring amino acid residues in peptides are abbreviated as recommended by the IUPAC-IUB Biochemical Nomenclature Commission as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Norleucine is Nle; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; Glycine is Gly or G, and X is any amino acid. Other naturally occurring amino acids include, by way of example, 4-hydroxyproline, 5-hydroxylysine, and the like.

Synthetic or non-naturally occurring amino acids refer to amino acids which do not naturally occur *in vivo* but which, nevertheless, can be incorporated into the peptide structures described herein. The resulting "synthetic peptide" contains amino acids other than the 20 naturally occurring, genetically encoded amino acids at one, two, or more positions of the peptides. For instance, naphthylalanine can be substituted for Tryptophan to facilitate synthesis. Other synthetic amino acids that can be substituted into peptides include L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxylysyl and D-alpha-methylalanyl, L-alpha.-methylalanyl, beta.-amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be incorporated into the peptides. Other derivatives include replacement of the

naturally occurring side chains of the 20 genetically encoded amino acids (or any L or D amino acid) with other side chains.

As used herein, the term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

A "polylinker" is a nucleic acid sequence that comprises a series of three or more closely spaced restriction endonuclease recognitions sequences.

As used herein, the term "purified" and like terms relate to an enrichment of a molecule or compound relative to other components normally associated with the molecule or compound in a native environment. The term "purified" does not necessarily indicate that complete purity of the particular molecule has been achieved during the process. A "highly purified" compound as used herein refers to a compound that is greater than 90% pure.

The determination of percent identity between two nucleotide or amino acid sequences can be accomplished using a mathematical algorithm. For example, a mathematical algorithm useful for comparing two sequences is the algorithm of Karlin and Altschul (1990, Proc. Natl. Acad. Sci. USA 87:2264-2268), modified as in Karlin and Altschul (1993, Proc. Natl. Acad. Sci. USA 90:5873-5877). This algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990, J. Mol. Biol. 215:403-410), and can be accessed, for example at the National Center for Biotechnology Information (NCBI) world wide web site having the universal resource locator "http://www.ncbi.nlm.nih.gov/BLAST/". BLAST nucleotide searches can be performed with the NBLAST program (designated "blastn" at the NCBI web site), using the following parameters: gap penalty = 5; gap extension penalty = 2; mismatch penalty = 3; match reward = 1; expectation value 10.0; and word size = 11 to obtain nucleotide sequences homologous to a nucleic acid described herein. BLAST protein searches can be performed with the XBLAST program (designated "blastn" at the NCBI web site) or the NCBI "blastp" program, using the following parameters: expectation value 10.0, BLOSUM62 scoring matrix to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped

BLAST can be utilized as described in Altschul et al. (1997, Nucleic Acids Res. 25:3389-3402). Alternatively, PSI-Blast or PHI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Id.) and relationships between molecules which share a common pattern. When utilizing BLAST, Gapped BLAST, PSI-Blast, and PHI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

"Primer" refers to a polynucleotide that is capable of specifically hybridizing to a designated polynucleotide template and providing a point of initiation for synthesis of a complementary polynucleotide. Such synthesis occurs when the polynucleotide primer is placed under conditions in which synthesis is induced, i.e., in the presence of nucleotides, a complementary polynucleotide template, and an agent for polymerization such as DNA polymerase. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers are useful for many applications. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers can be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

A "subject" of diagnosis or treatment is a mammal, including a human. Non-human mammals subject to diagnosis or treatment include, but are not limited to, for example, primates, horses, cows, sheep, cats, and dogs.

A "test" cell is a cell being examined.

A "therapeutically effective amount" of a compound is that amount of compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

As used herein, a "transgenic cell" is any cell that comprises a nucleic acid sequence that has been introduced into the cell in a manner that allows expression of a gene encoded by the introduced nucleic acid sequence.

A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses that incorporate the recombinant polynucleotide.

Embodiments

In accordance with one embodiment of the present invention proteins associated with the lipid raft domains of sperm cells are isolated and characterized.

One aspect of the present invention relates to signaling events in mammalian sperm that regulate the functions of this highly differentiated cell. More particularly, in one embodiment the invention relates to signal transduction that modulates the acquisition of sperm fertilizing capacity.

One embodiment of the present invention is directed to the mouse and human Band 5 proteins that are testis abundant and expressed predominantly if not exclusively in the male germ cells of humans and mice. More particularly the present invention is directed to mouse and human Band 5 and the use of that protein to prepare and isolate compounds that can be used as diagnostic and contraceptive agents. One of ordinary skill in the art will appreciate that the invention also included other mammalian homologs of Band 5, as well as derivatives, fragments, and modifications thereof.

The association of this unique protein with the raft membrane domains has

led applicants to believe that this protein is relevant to capacitation and/or sperm/egg binding, and thus the Band 5 gene and protein product represent potential targets for of contraceptive agents. Accordingly, one aspect of the present invention is directed to the isolation of human Band 5 and its use in isolating agents that inhibit capacitation-associated tyrosine phosphorylation. Such inhibitors can then be used as contraceptive agents to inhibit fertilization. In accordance with one embodiment, the Band 5 proteins will be used to screen for specific inhibitors of Band 5 activity and these inhibitors will be used either alone or in conjunction with other contraceptive agents to prevent unintended pregnancies.

In accordance with one embodiment of the present invention a purified polypeptide is provided comprising the amino acid sequence of mouse or human Band 5 (as shown if Fig. 6), or an amino acid sequence that differs from those sequences by one or more conservative amino acid substitutions. In another embodiment the purified polypeptide comprises an amino acid sequence that differs from those of Fig. 6 by less than 5 conservative amino acid substitutions, and in a further embodiment, by 2 or less conservative amino acid substitutions. In one embodiment the purified polypeptide comprises the amino acid sequence of Fig. 6.

In one embodiment, the polypeptide of the invention has at least 30% sequence identity with SEQ ID NO:7 or 8. In one aspect, the polypeptide of the invention has at least 40% sequence identity with SEQ ID NO:7 or 8. In another aspect, the polypeptide of the invention has at least 50% sequence identity with SEQ ID NO:7 or 8. In another aspect, the polypeptide of the invention has at least 60% sequence identity with SEQ ID NO:7 or 8. In yet another aspect, the polypeptide of the invention has at least 70% sequence identity with SEQ ID NO:7 or 8. In a further aspect, the polypeptide of the invention has at least 80% sequence identity with SEQ ID NO:7 or 8. In another aspect, the polypeptide of the invention has at least 90% sequence identity with SEQ ID NO:7 or 8.

The polypeptides of the present invention may include additional amino acid sequences to assist in the stabilization and/or purification of recombinantly produced polypeptides. These additional sequences may include intra- or inter-cellular targeting peptides or various peptide tags known to those skilled in the art. In one embodiment, the purified polypeptide comprises an amino acid sequence selected from Fig. 6 and a peptide tag. Suitable expression vectors for expressing such fusion proteins and suitable peptide tags are known to those skilled in the art and are

commercially available. In one embodiment the tag comprises a His tag. In another embodiment, the present invention is directed to a purified bioactive polypeptide that comprises a portion of a polypeptide of Fig. 6.

The polypeptides of the present invention may be readily prepared by standard, well-established techniques, such as solid-phase peptide synthesis (SPPS) as described by Stewart et al. in Solid Phase Peptide Synthesis, 2nd Edition, 1984, Pierce Chemical Company, Rockford, Illinois; and as described by Bodanszky and Bodanszky in The Practice of Peptide Synthesis, 1984, Springer-Verlag, New York. At the outset, a suitably protected amino acid residue is attached through its carboxyl group to a derivatized, insoluble polymeric support, such as cross-linked polystyrene or polyamide resin. "Suitably protected" refers to the presence of protecting groups on both the α-amino group of the amino acid, and on any side chain functional groups. Side chain protecting groups are generally stable to the solvents, reagents and reaction conditions used throughout the synthesis, and are removable under conditions which will not affect the final peptide product. Stepwise synthesis of the oligopeptide is carried out by the removal of the N-protecting group from the initial amino acid, and couple thereto of the carboxyl end of the next amino acid in the sequence of the desired peptide. This amino acid is also suitably protected. The carboxyl of the incoming amino acid can be activated to react with the N-terminus of the support-bound amino acid by formation into a reactive group such as formation into a carbodiimide, a symmetric acid anhydride or an "active ester" group such as hydroxybenzotriazole or pentafluorophenyl esters.

Examples of solid phase peptide synthesis methods include the BOC method which utilized tert-butyloxcarbonyl as the α-amino protecting group, and the FMOC method which utilizes 9-fluorenylmethyloxcarbonyl to protect the α-amino of the amino acid residues, both methods of which are well-known by those of skill in the art.

Incorporation of N- and/or C- blocking groups can also be achieved using protocols conventional to solid phase peptide synthesis methods. For incorporation of C-terminal blocking groups, for example, synthesis of the desired peptide is typically performed using, as solid phase, a supporting resin that has been chemically modified so that cleavage from the resin results in a peptide having the desired C-terminal blocking group. To provide peptides in which the C-terminus bears a primary amino blocking group, for instance, synthesis is performed using a

p-methylbenzhydrylamine (MBHA) resin so that, when peptide synthesis is completed, treatment with hydrofluoric acid releases the desired C-terminally amidated peptide. Similarly, incorporation of an N-methylamine blocking group at the C-terminus is achieved using N-methylaminoethyl-derivatized DVB, resin, which upon HF treatment releases a peptide bearing an N-methylamidated C-terminus. Blockage of the C-terminus by esterification can also be achieved using conventional procedures. This entails use of resin/blocking group combination that permits release of side-chain peptide from the resin, to allow for subsequent reaction with the desired alcohol, to form the ester function. FMOC protecting group, in combination with DVB resin derivatized with methoxyalkoxybenzyl alcohol or equivalent linker, can be used for this purpose, with cleavage from the support being effected by TFA in dicholoromethane. Esterification of the suitably activated carboxyl function e.g. with DCC, can then proceed by addition of the desired alcohol, followed by deprotection and isolation of the esterified peptide product.

Incorporation of N-terminal blocking groups can be achieved while the synthesized peptide is still attached to the resin, for instance by treatment with a suitable anhydride and nitrile. To incorporate an acetyl blocking group at the N-terminus, for instance, the resin-coupled peptide can be treated with 20% acetic anhydride in acetonitrile. The N-blocked peptide product can then be cleaved from the resin, deprotected and subsequently isolated.

To ensure that the peptide obtained from either chemical or biological synthetic techniques is the desired peptide, analysis of the peptide composition should be conducted. Such amino acid composition analysis may be conducted using high resolution mass spectrometry to determine the molecular weight of the peptide. Alternatively, or additionally, the amino acid content of the peptide can be confirmed by hydrolyzing the peptide in aqueous acid, and separating, identifying and quantifying the components of the mixture using HPLC, or an amino acid analyzer. Protein sequenators, which sequentially degrade the peptide and identify the amino acids in order, may also be used to determine definitely the sequence of the peptide.

Prior to its use, the peptide is purified to remove contaminants. In this regard, it will be appreciated that the peptide will be purified so as to meet the standards set out by the appropriate regulatory agencies. Any one of a number of a conventional purification procedures may be used to attain the required level of

purity including, for example, reversed-phase high-pressure liquid chromatography (HPLC) using an alkylated silica column such as C_4 -,C_8- or C_{18}- silica. A gradient mobile phase of increasing organic content is generally used to achieve purification, for example, acetonitrile in an aqueous buffer, usually containing a small amount of trifluoroacetic acid. Ion-exchange chromatography can be also used to separate peptides based on their charge.

The present invention also encompasses isolated nucleic acids comprising nucleic acid sequences that encode the polypeptides of Fig. 6, as well as homologs, derivatives, fragments, and modifications thereof. Primers were designed to an internal 500bp segment of the mouse testis cDNA for Band 5. A gene specific product was obtained by PCR. Using the gene specific primers and RACE extension of marathon ready mouse testis cDNA (ClonTech) the complete 1.5 kb insert was cloned and sequenced. The present invention is also directed to recombinant human Band 5 gene constructs. In one embodiment, the recombinant gene construct comprises a non-native promoter operably linked to a nucleic acid sequence encoding the polypeptide of Fig. 6. The non-native promoter is preferably a strong constitutive promoter that allows for expression in a predetermined host cell. These recombinant gene constructs can be introduced into host cells to produce transgenic cell lines that synthesize the Band 5 protein. Host cells can be selected from a wide variety of eukaryotic and prokaryotic organisms, and two preferred host cells are *E. coli* and yeast cells.

In one embodiment, the introduced nucleic acid is sufficiently stable in the transgenic cell (i.e. incorporated into the cell's genome, or present in a high copy plasmid) to be passed on to progeny cells. The cells can be propagated *in vitro* using standard cell culture procedure, or in an alternative embodiment, the host cells are eukaryotic cells and are propagated as part of an animal, including for example, a transgenic animal. In one embodiment the transgenic cell is a human cell and comprises a nucleic acid sequence encoding the human Band 5 protein.

It is not intended that the present invention be limited by the nature of the nucleic acid employed. The target nucleic acid may be native or synthesized nucleic acid. The nucleic acid may be from a viral, bacterial, animal or plant source. The nucleic acid may be DNA or RNA and may exist in a double-stranded, single-stranded, or partially double-stranded form. Furthermore, the nucleic acid may be found as part of a virus or other macromolecule. See, e.g., Fasbender et al., 1996, J.

Biol. Chem. 272:6479-89 (polylysine condensation of DNA in the form of adenovirus).

Nucleic acids useful in the present invention include, by way of example and not limitation, oligonucleotides and polynucleotides such as antisense DNAs and/or RNAs; ribozymes; DNA for gene therapy; viral fragments including viral DNA and/or RNA; DNA and/or RNA chimeras; mRNA; plasmids; cosmids; genomic DNA; cDNA; gene fragments; various structural forms of DNA including single-stranded DNA, double-stranded DNA, supercoiled DNA and/or triple-helical DNA; Z-DNA; and the like. The nucleic acids may be prepared by any conventional means typically used to prepare nucleic acids in large quantity. For example, DNAs and RNAs may be chemically synthesized using commercially available reagents and synthesizers by methods that are well-known in the art (see, e.g., Gait, 1985, OLIGONUCLEOTIDE SYNTHESIS: A PRACTICAL APPROACH (IRL Press, Oxford, England)). RNAs may be produce in high yield via in vitro transcription using plasmids such as SP65 (Promega Corporation, Madison, WI).

In some circumstances, as where increased nuclease stability is desired, nucleic acids having modified internucleoside linkages may be preferred. Nucleic acids containing modified internucleoside linkages may also be synthesized using reagents and methods that are well known in the art. For example, methods for synthesizing nucleic acids containing phosphonate phosphorothioate, phosphorodithioate, phosphoramidate methoxyethyl phosphoramidate, formacetal, thioformacetal, diisopropylsilyl, acetamidate, carbamate, dimethylene-sulfide (-CH2-S-CH2), diinethylene-sulfoxide (-CH2-SO-CH2), dimethylene-sulfone (-CH2-SO2-CH2), 2'-O-alkyl, and 2'-deoxy2'-fluoro phosphorothioate internucleoside linkages are well known in the art (see Uhlmann et al., 1990, Chem. Rev. 90:543-584; Schneider et al., 1990, Tetrahedron Lett. 31:335 and references cited therein).

The nucleic acids may be purified by any suitable means, as are well known in the art. For example, the nucleic: acids can be purified by reverse phase or ion exchange HPLC, size exclusion chromatography or gel electrophoresis. Of course, the skilled artisan will recognize that the method of purification will depend in part on the size of the DNA to be purified.

The term nucleic acid also specifically includes nucleic acids composed of bases other than the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil).

Modified gene sequences, i.e. genes having sequences that differ from the gene sequences encoding the naturally-occurring proteins, are also encompassed by the invention, so long as the modified gene still encodes a protein that functions to stimulate healing in any direct or indirect manner. These modified gene sequences include modifications caused by point mutations, modifications due to the degeneracy of the genetic code or naturally occurring allelic variants, and further modifications that have been introduced by genetic engineering, i.e., by the hand of man.

Techniques for introducing changes in nucleotide sequences that are designed to alter the functional properties of the encoded proteins or polypeptides are well known in the art. Such modifications include the deletion, insertion, or substitution of bases, and thus, changes in the amino acid sequence. Changes may be made to increase the activity of a protein, to increase its biological stability or half-life, to change its glycosylation pattern, and the like. All such modifications to the nucleotide sequences encoding such proteins are encompassed by this invention.

The present invention also encompasses a method for producing human or mouse Band 5. The method comprises the steps of introducing a nucleic acid sequence comprising a sequence that encodes human or mouse Band 5 into a host cell, and culturing the host cell under conditions that allow for expression of the introduced human Band 5 gene. In one embodiment the promoter is a conditional or inducible promoter, alternatively the promoter may be a tissue specific or temporal restricted promoter (i.e. operably linked genes are only expressed in a specific tissue or at a specific time). The synthesized Band 5 proteins can be purified using standard techniques and used in high throughput screens to identify compounds that bind to Band 5 under physiological relevant conditions and/or that inhibit capacitation associated phosphorylation of tyrosine residues of sperm proteins.

Alternatively, in one embodiment the recombinantly produced Band 5 polypeptides, or homologs, fragments, derivatives, or modifications thereof are used to generate antibodies against the Band 5 polypeptides. The recombinantly produced Band 5 proteins can also be used to obtain crystal structures. Such structures would allow for crystallography analysis that would lead to the design of specific drugs to inhibit Band 5 function.

In accordance with one embodiment a composition is provided comprising a purified peptide of Fig. 6, or an antigenic fragment thereof. In one embodiment the

peptide consists of the sequence of Fig. 6. The compositions can be combined with a pharmaceutically acceptable carrier or adjuvants and administered to a mammalian species to induce an immune response.

Another embodiment of the present invention is directed to antibodies specific for human or mouse Band 5. In one embodiment the antibody is a monoclonal antibody. The antibodies or antibody fragments of the present invention can be combined with a carrier or diluent to form a composition. In one embodiment, the carrier is a pharmaceutically acceptable carrier. Such carriers and diluents include sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically and physiologically acceptable carrier, including adjuvants, excipients or stabilizers. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose, and related sugar solution, and glycols such as, propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions.

Antibodies to Band 5 polypeptides may be generated using methods that are well known in the art. In accordance with one embodiment an antibody is provided that specifically binds to a polypeptide selected from Fig. 6 or an antigenic fragment of a homolog thereof. The antibodies may be used with or without modification, and may be labeled by joining them, either covalently or non-covalently, with a reporter molecule. In addition, the antibodies can be formulated with standard carriers and optionally labeled to prepare therapeutic or diagnostic compositions.

In accordance with on embodiment of the invention and antibody is provided that specifically binds to the peptide sequence SMVGPEDAGNYRC. This peptide is unique in the database for mouse Band 5. No other proteins match exactly to this sequence and the sequence is highly conserved between the mouse and human Band 5 sequences. This unique 13 amino acid sequence in the nonordinary secondary structure domain in Band 5. Results of a preliminary antibody screen are shown in Fig. 9. One specific band is detected at about 100 kDa whereas the expected MW is 45 kDa. The preimmune and secondary antibody controls did not produce any significant signal.

One of ordinary skill in the art will appreciate that other antibodies can also be prepared against other sequences or fragments of the polypeptides of the invention. In one aspect, the antibody of the invention is directed against a sequence

of SEQ ID NO:8. In one aspect, the antibody is inhibitory to the function of the polypeptide. In another aspect, the antibody is useful for diagnostic or therapeutic purposes.

The generation of polyclonal antibodies is accomplished by inoculating the desired animal with the antigen and isolating antibodies which specifically bind the antigen therefrom.

Monoclonal antibodies directed against full length or peptide fragments of a protein or peptide may be prepared using any well known monoclonal antibody preparation procedures, such as those described, for example, in Harlow et al. (1988, In: Antibodies, A Laboratory Manual, Cold Spring Harbor, NY) and in Tuszynski et al. (1988, Blood, 72:109-115). Quantities of the desired peptide may also be synthesized using chemical synthesis technology. Alternatively, DNA encoding the desired peptide may be cloned and expressed from an appropriate promoter sequence in cells suitable for the generation of large quantities of peptide. Monoclonal antibodies directed against the peptide are generated from mice immunized with the peptide using standard procedures as referenced herein.

Nucleic acids encoding the monoclonal antibody obtained using the procedures described herein may be cloned and sequenced using technology which is available in the art, and is described, for example, in Wright et al. (1992, Critical Rev. in Immunol. 12(3,4):125-168) and the references cited therein. Further, the antibody of the invention may be "humanized" using the technology described in Wright et al., (supra) and in the references cited therein, and in Gu et al. (1997, Thrombosis and Hematocyst 77(4):755-759).

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells, e.g., the hybridoma, which express the desired protein to be expressed on the phage surface, e.g., the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies immunoglobulin fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying immunoglobulin genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY).

Bacteriophage which encode the desired antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, e.g., the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell. Such panning techniques are well known in the art and are described for example, in Wright et al., (supra).

Processes such as those described above, have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab immunoglobulin. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human immunoglobulin rather than cells which express human immunoglobulin.

The procedures just presented describe the generation of phage which encode the Fab portion of an antibody molecule. However, the invention should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFv/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage libraries comprising scFv DNA may be generated following the procedures described in Marks et al., 1991, J. Mol. Biol. 222:581-597. Panning of phage so generated for the isolation of a desired antibody is conducted in a manner similar to that described for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities (Barbas, 1995, Nature Medicine 1:837-839; de Kruif et al. 1995, J. Mol. Biol.248:97-105).

Since Band 5 is demonstrated herein to be highly testis abundant (See Figs 5-7), this makes Band 5 an optimal target for the development of drugs that modulate its activity to study Band 5's role in spermiogenesis. Furthermore, inhibitors of Band 5 activity are anticipated to have utility as contraceptive agents. In accordance with one aspect of the present invention the Band 5 protein is used as a target for the development of novel drugs. Progress in the field of small molecule library generation, using combinatorial chemistry methods coupled to high-throughput screening, has accelerated the search for ideal cell-permeable inhibitors. In addition, structural-based design using crystallographic methods has improved the ability to characterize in detail ligand-protein interaction sites that can be exploited for ligand design.

In one embodiment, the present invention provides methods of screening for agents, small molecules, or proteins that interact with polypeptides comprising the sequences of Fig. 6 or bioactive fragments thereof. The invention encompasses both *in vivo* and *in vitro* assays to screen small molecules, compounds, recombinant proteins, peptides, nucleic acids, antibodies *etc.* which bind to or modulate the activity of Band 5 and are thus useful as therapeutic or diagnostic markers for fertility. In addition to the assays disclosed herein, other assays for screening agents which interact with polypeptides of the invention are available and are known to those of ordinary skill in the art.

In one embodiment of the present invention Band 5 polypeptides for Fig. 6 are used to isolate ligands that bind to Band 5 under physiological conditions. The screening method comprises the steps of contacting a Band 5 polypeptide with a mixture of compounds under physiological conditions, removing unbound and non-specifically bound material, and isolating the compounds that remain bound to the tssk polypeptide. Typically, the Band 5 polypeptide will be bound to a solid support, using standard techniques, to allow for rapid screening of compounds. The solid support can be selected from any surface that has been used to immobilize biological compounds and includes but is not limited to polystyrene, agarose, silica or nitrocellulose. In one embodiment the solid surface comprises functionalized

silica or agarose beads. Screening for such compounds can be accomplished using libraries of pharmaceutical agents and standard techniques known to the skilled practitioner.

Ligands or agents that bind to the Band 5 polypeptides can then be further analyzed for agonists and antagonists activity through the use of an *in vitro* kinase assay. Inhibitors of Band 5 associated kinase activity have potential use as agents that prevent maturation/capacitation of sperm. In accordance with one embodiment, inhibitors of Band 5 are isolated as potential contraceptive agents. Such inhibitors can be formulated as pharmaceutical compositions and administered to a subject to block spermatogenesis and provide a means for contraception.

Band 5 polypeptides of the invention are screened in the presence or absence of a test compound and activity of the polypeptide is determined. A higher or lower level of activity in the presence of the test compound, compared with the level of activity in the absence of the test compound, is an indication that the test compound affects activity of the Band 5 polypeptide of the invention.

Band 5 polypeptides, or regulators of Band 5 gene or protein expression or activity may be administered to animals in need thereof.

The invention also encompasses the use of pharmaceutical compositions of an appropriate Band 5 polypeptide, or agent which regulates Band 5 gene and protein expression or activity, to practice the methods of the invention, said compositions further comprising a pharmaceutically-acceptable carrier.

The pharmaceutical compositions useful for practicing the invention may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In one embodiment, the invention envisions administration of a dose which results in a concentration of between 1 μM and 10 μM in a tissue of a mammal.

Pharmaceutical compositions that are useful in the methods of the invention may be administered systemically in oral solid formulations, ophthalmic, suppository, aerosol, topical or other similar formulations. In addition to the appropriate hypericin derivative, such pharmaceutical compositions may contain pharmaceutically-acceptable carriers and other ingredients known to enhance and facilitate drug administration. Other possible formulations, such as nanoparticles, liposomes, resealed erythrocytes, and immunologically based systems may also be used to administer an appropriate hypericin derivative according to the methods of the invention.

Compounds which are identified using any of the methods described herein may be formulated and administered to a mammal for treatment of the diseases or disorder disclosed herein, or as a method of contraception, are now described.

The invention encompasses the preparation and use of pharmaceutical compositions comprising a compound useful for treatment of the diseases disclosed herein as an active ingredient. Such a pharmaceutical composition may consist of the active ingredient alone, in a form suitable for administration to a subject, or the pharmaceutical composition may comprise the active ingredient and one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The active ingredient may be present in the pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

As used herein, the term "pharmaceutically acceptable carrier" means a chemical composition with which the active ingredient may be combined and which, following the combination, can be used to administer the active ingredient to a subject.

As used herein, the term "physiologically acceptable" ester or salt means an ester or salt form of the active ingredient which is compatible with any other ingredients of the pharmaceutical composition, which is not deleterious to the subject to which the composition is to be administered.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and

perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the invention is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs, birds including commercially relevant birds such as chickens, ducks, geese, and turkeys.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, intrathecal or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active agents. Particularly contemplated additional agents include anti-emetics and scavengers such as cyanide and cyanate scavengers.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet,

a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture.

Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycollate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a

sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include, for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (e.g. polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol

monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl para hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil in water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan

monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (i.e. about 20°C) and which is liquid at the rectal temperature of the subject (i.e. about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into the structure of a material during the synthesis of the material (i.e. such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier.

As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen free water) prior to parenteral administration of the reconstituted composition.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents,

wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non toxic parenterally acceptable diluent or solvent, such as water or 1,3 butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi liquid preparations such as liniments, lotions, oil in water or water in oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a

solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods,

and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1 1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other opthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in Genaro, ed., 1985, Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

Typically, dosages of the compound of the invention which may be administered to an animal, preferably a human, range in amount from 1 μg to about 100 g per kilogram of body weight of the animal. While the precise dosage administered will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the

animal and the route of administration. Preferably, the dosage of the compound will vary from about 1 mg to about 10 g per kilogram of body weight of the animal. More preferably, the dosage will vary from about 10 mg to about 1 g per kilogram of body weight of the animal.

The compound may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even lees frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

The invention also provides for the preparation and use of transgenic non-human mammals comprising nucleic acid sequences of the invention. Precise protocols for the generation of transgenic mice are disclosed in Nagy and Rossant (1993, In: Gene Targeting, A Practical Approach, Joyner ed. IRL Press, pp. 146-179). and are therefore not repeated herein. Transfection or transduction of ES cells in order to introduce the desired DNA therein is accomplished using standard protocols, such as those described, for example, in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in Ausubel et al. (1997, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Preferably, the desired DNA contained within the transgene of the invention is electroporated into ES cells, and the cells are propagated as described in Soriano et al. (1991, Cell 64:693-702).

The transgenic mammal of the invention can be any species of non-human mammal. Thus, the invention should be construed to include generation of transgenic mammals encoding the chimeric nucleic acid, which mammals include mice, hamsters, rats, rabbits, pigs, sheep and cattle. The methods described herein for generation of transgenic mice can be analogously applied using any mammalian species. Preferably, the transgenic mammal of the invention is a rodent and even more preferably, the transgenic mammal of the invention is a mouse.

To identify the transgenic mammals of the invention, pups are examined for the presence of the isolated nucleic acid using standard technology such as Southern blot hybridization, PCR, and/or RT-PCR, or the presence of the protein or peptide can be detected by techniques known to those of skill in the art.

Alternatively, recombinant cells expressing a polypeptide of the invention can be administered in *ex vivo* and *in vivo* therapies where administering the recombinant cells thereby administers the protein to a cell, a tissue, and/or an animal. Additionally, the recombinant cells are useful for the discovery of Band 5 domain signaling pathways.

Those of skill in the art will appreciate that other uses exist for recombinant cells and transgenic animals.

The invention also includes a kit comprising the composition of the invention and an instructional material which describes adventitially administering the composition to a cell or a tissue of a mammal. In another embodiment, this kit comprises a (preferably sterile) solvent suitable for dissolving or suspending the composition of the invention prior to administering the compound to the mammal. As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the peptide of the invention in the kit for effecting alleviation of the various diseases or disorders recited herein. Optionally, or alternately, the instructional material may describe one or more methods of alleviation the diseases or disorders in a cell or a tissue of a mammal. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the peptide of the invention or be shipped together with a container which contains the peptide. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

Examples

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Raft fractions can be isolated with reproducibility from mouse caudal sperm using ultracentrifugation of membranes in a sucrose gradient according to standard techniques known to those skilled in the art (see Fig. 1). As shown in Fig. 2, the proteins present in sucrose fractions of the isolated lipid raft domains isolated from

noncapacitated sperm differ from those isolated from capacitated sperm. Silver stained PAGE analysis reveals that a number of proteins present in the lipid raft domains of noncapacitated sperm are not present in those domains in capacitated sperm, thus raft domains are diminished in protein content (especially true for fraction 4) upon capacitation of the sperm (see Fig. 2).

Proteins residing in fraction 4 of the raft plasma membrane domain of noncapacitated mouse sperm were isolated and submitted for mass spec peptide analysis. Five peptide sequences were identified by mass spec analysis of an approximately 60 kDa protein recovered from raft fraction number 4 and had the following sequences:

25-34 CDQFVTDALK (SEQ ID NO:1)
192-200 GLTDYSFYR (SEQ ID NO:2)
221-232 SMVGPEDAGNYRC (SEQ ID NO:3)
233-247 CVLDTINQGHATVIR (SEQ ID NO:4)
351-364 NASDEVKPTASGSK (SEQ ID NO:5)

The mouse Band5 nucleic acid sequence (SEQ ID NO:6), accession number AK006830, is:

gggatgaccggtgactcttggagtcagtagaaaaaccctaaattactctccccagtcttccaaggaaaaccttactcttagt
cgtgagaaagtattttaaaaagtcctcagaacctggactgagctgggtgaagcattagcgcttgagggcgcggaattgga
aagcggcttttaaagggtgttgtttaagcgccctccccccctgccctctgtttggggtgagaggatcctctggcggtgctgg
caagctgtgcgttctggccttcatggggccgcattttacactcttgctggcagctcttgccaactgcctgtgtccagggagg
ccctgcatcaaatgtgaccagtttgtgacagatgcgctaaagactttcgaaaacacttacctgaatgaccacctgccacac
gacattcacaaaaatgtaatgaggatggtgaaccatgaagtatcgagcttcggcgtagtcacttcggctgaggattcctatt
tgggggccgtggacgagaacacactggaacaagcaacctggagttttctgaaggatctgaagcgtattacagacagtga
cttaaaaggagagctctttataaaggaactattgtggatgcttcgtcatcaaaaggacatctttaacaatcttgctagacagtt
ccaaaaggaagttctttgtcccaacaaatgcggagtgatgtcgcagactttgatctggtgtcttaagtgcgaaaagcagttg
cacatttgtcggaaatccctagattgtggagagcgccacatagaggtacatcgctcggaagacctggtcctggactgtct
gctcagttggcatcgtgcttctaagggacttacagattacagtttttacagggtttgggagaacagttctgagaccttgattg
ccaaggggaaagaaccatatctgaccaagtcgatggtgggtccagaggatgctggcaactaccgctgtgtgctagatac
catcaaccaaggtcatgccaccgtcatccgctacgatgtcacagtattgccccccaaagcattcagaggaaaaccaacca
ccgaacatcataacccaagaggagcacgagactcctgtccacgtgactccacagacaccaccggggcaggagccag
agtcggagctgtacccggagctgcacccagagttgtacccggagctcatacctacagtcgctcaaaatccagagaaaaa

aatgaaaactcgcctcttaatactgttgacccttggctttgtggttcttgtggccagcatcattatctcggtacttcactttagga
aagtcagcgctaaattgaagaatgcaagtgacgaagtcaaacccaccgcgtcaggatccaagtcagatcagagtttgtc
ccaacagatgggattgaagaaggcctctcaggcagattttaactctgactactctggagataagagcgaggcaacagaa
aactaataaagatttgtatttgactc.

The mouse Band5 protein amino acid sequence (SEQ ID NO:7), accession number BAB24761.1, is:

MGPHFTLLLA ALANCLCPGR PCIKCDQFVT DALKTFENTY LNDHLPHDIH
KNVMRMVNHE VSSFGVVTSA EDSYLGAVDE NTLEQATWSF
LKDLKRITDS DLKGELFIKE LLWMLRHQKD IFNNLARQFQ KEVLCPNKCG
VMSQTLIWCL KCEKQLHICR KSLDCGERHI EVHRSEDLVL DCLLSWHRAS
KGLTDYSFYR VWENSSETLI AKGKEPYLTK SMVGPEDAGN
YRCVLDTINQ GHATVIRYDV TVLPPKHSEE NQPPNIITQE EHETPVHVTP
QTPPGQEPES ELYPELHPEL YPELIPTVAQ NPEKKMKTRL LILLTLGFVV
LVASIIISVL HFRKVSAKLK NASDEVKPTA SGSKSDQSLS QQMGLKKASQ
ADFNSDYSGD KSEATEN.

The human Band 5 protein amino acid sequence (SEQ ID NO:8; NCBI GenBank Accession No. NM_182575) disclosed herein, comprising 350 amino residues, comprises the sequence:

MGPHFTLLCAALAGCLLPAEGCVICDPSVVLALKSLEKDYLPGHLDAKHHK
AMMERVENAVKDFQELSLNEDAYMGVVDEATLQKGSWSLLKDLKRITDS
DVKGDLFVKELFWMLHLQKETFATYVARFQKEAYCPNKCGVMLQTLIWC
KNCKKEVHACRKSYDCGERNVEVPQMEDMILDCELNWHQASEGLTDYSFY
RVWGNNTETLVSKGKEATLTKPMVGPEDAGSYRCELGSVNSSPATIINFHV
TVLPKMIKEEKPSPNIVTPGEATTESSISLQPLQPEKMLASRLLGLLICGSLALI
TGLTFAIFRRRKVIDFIKSSLFGLGSGVAEQTQVPKEKATDSRQQ.

The nucleic acid sequence (SEQ ID NO:9; NCBI GenBank Accession No. NM_182575) disclosed herein encoding human Band 5 protein (SEQ ID NO:8) comprises the sequence:

ggaatatctataaaggaattcgggcccctacgtgagaagctcggaggggggtggtttcaggagttccttgtggagagggg
atcgtccgtgactcagaccccgtcgaaaagctctcgatttcacgcaaccaaccttaccctcgtccccaacacaatccttga
ttttaccccctgggaaaattagcgtaggtggtcatgaccccggcttcctctttaaaagccctcagaacctggaccgggctcct

caatgaaccctagtgttagaggccacaaatcgggaatcaaggtcggggagcggctgtttagggtgcatcactgttcagg
atgcatcggggctccttttttctaagcggaagtgtgcgccctgcaaattggaagcccgttagttttggggaagggtgaacaa
gggcccccctaggtctagaggaccgcatggagtctcgcttaagtgttagtggggtcctcagttctagaaatctgcgttctcct
gctctcggtttggggtgagagcacccttcctaacggtcttcgggaactgtgcgcgccggctgcaatggggccgcatttta
ccctcctgtgtgcggcgctggccggttgcttgcttcctgccgaggggtgtgttatatgtgacccgtctgtcgtgctggcgct
aaagtccctggagaaagattacctgcctggccacctggatgcgaagcatcacaaagccatgatggaaagggtagagaa
tgccgtgaaggatttccaggaactgtcgcttaatgaggatgcctatatgggggtcgttgatgaggccacactgcaaaagg
ggtcctggagtttgctgaaggatctgaaacgcatcacagacagtgatgtaaaaggcgatctctttgtgaaggagctattttg
gatgttgcacttgcaaaaggaaacctttgccacctatgttgctcgattccaaaaagaggcttattgtcccaacaaatgtggt
gtgatgttgcaaactctgatctggtgcaagaactgcaaaaaggaggttcacgcttgtcgaaagtcctacgattgcgggga
gcggaatgtggaagttcctcaaatggaagacatgatcctggactgtgagttaaactggcatcaggcttcggaaggcctca
ctgattacagcttttacagggtttgggggaacaatacggagaccttggtgtccaaggggaaagaggccaccctgaccaa
gcccatggtgggtccagaggatgcaggcagctaccgctgcgagctgggctctgtgaattccagcccagccacgatcat
caattttcacgtcacagtgttgcccaaaatgatcaaggaggaaaaaccttctccaaatatcgtaaccccgggggaggcga
ccacggagtcgtccataagcctccagcctctgcagcccgagaaaatgctggcaagccgccttctggggctgctgatctg
cggctccctagcactgataaccggccttacctttgcgatatttcgtcgaaggaaggtgatcgatttcatcaaatcctcactgt
ttggccttggcagtggagttgccgagcaaacccaggtcccaaaagaaaaggccacagattcgaggcaacaataaagat
ttatcttgttgtctaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
aaaaaaaaaa.

An EST/cDNA search of the existing databases revealed only one protein that contained all five fragments (See Fig. 3) and the numbers to the left of the peptide fragments indicate the location of the peptide in the identified mouse protein. Further protein and nucleic acid blast analysis identified only one hypothetical human ortholog that matched with a significant E value to the mouse band 5 protein. A summary of the bioinformatics information generated for both the human (SEQ ID NO:8) and mouse (SEQ ID NO:7) Band 5 protein is provided in Figs. 4 & 5. An alignment of the mouse (SEQ ID NO:7) and human (SEQ ID NO:8) Band 5 sequences reveals the two proteins share a high degree of sequence identity (See Fig. 6).

Real time PCR was conducted using primers that were designed to produce a product less than 200 bp in length. An initial run was done on mouse testis quick-clone cDNA (ClonTech) using G3PHD primers as a positive control and water as a negative control. As shown in Fig. 7, a single product was produced as indicated by the melt curve and by agarose gel analysis. Real time PCR was then conducted

using a mouse multiple tissue cDNA panel screen. The results are shown in Fig. 8 and indicate that the Band 5 transcript is highly expressed in testis.

To determine if the Band 5 protein is involved in sperm capacitation an experiment was conducted using Western blot analysis of sperm proteins to detect capacitation-associated tyrosine phosphorylation of sperm proteins under varying conditions. In particular proteins were isolated from sperm cells under four separate conditions:

1. Noncapacitated Sperm
2. Capacitated Sperm-1 Hour @ 37°
3. Capacitated Sperm in the Presence of a 1:50 Dilution Of Immune Sera
4. Capacitated Sperm in the Presence of a 1:50 Dilution Of Pre-Immune Sera

The results are shown in Fig. 9. The immune sera specifically detected a 100 kDa protein band. Two sets of mouse sperm proteins were used for this experiment. Both sets indicate that the antibody generated against the peptide sequence SMVGPEDAGNYRC reduced the amount of tyrosine phosphorylation of sperm proteins during capacitation (see Fig. 10). Without wishing to be bound by any particular theory, the data indicate that Raft proteins play key roles in capacitation and are potential contraceptive targets.

The disclosures of each and every publication cited herein are hereby incorporated herein by reference in their entirety.

Other methods which were used but not described herein are well known and within the competence of one of ordinary skill in the art of clinical, chemical, cellular, histochemical, biochemical, molecular biology, microbiology and recombinant DNA techniques. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.